Examining *Cuphea* as a Potential Host for Western Corn Rootworm (Coleoptera: Chrysomelidae): Larval Development

ROBERT W. BEHLE,[1] BRUCE E. HIBBARD,[2] STEVEN C. CERMAK,[3] AND TERRY A. ISBELL[3]

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ABSTRACT In previous crop rotation research, adult emergence traps placed in plots planted to *Cuphea* PSR-23 (a selected cross of *Cuphea viscosissma* Jacq. and *Cuphea lanceolata* Ait.) caught high numbers of adult western corn rootworms, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), suggesting that larvae may have completed development on this broadleaf plant. Because of this observation, a series of greenhouse and field experiments were conducted to test the hypothesis that *Cuphea* could serve as a host for larval development. Greenhouse-grown plants infested with neonates of a colonized nondiapausing strain of the beetle showed no survival of larvae on *Cuphea*, although larvae did survive on the positive control (corn, *Zea mays* L.) and negative control [sorghum, *Sorghum bicolor* (L.) Moench] plants. Soil samples collected 20 June, 7 July, and 29 July 2005 from field plots planted to *Cuphea* did not contain rootworm larvae compared with means of 1.28, 0.22, and 0.00 rootworms kg^{-1} soil, respectively, for samples collected from plots planted to corn. Emergence traps captured a peak of eight beetles $trap^{-1}$ day^{-1} from corn plots on 8 July compared with a peak of 0.5 beetle $trap^{-1}$ day^{-1} on 4 August from *Cuphea* plots. Even though a few adult beetles were again captured in the emergence traps placed in the *Cuphea* plots, it is not thought to be the result of successful larval development on *Cuphea* roots. All the direct evidence reported here supports the conventional belief that rootworm larvae do not survive on broadleaf plants, including *Cuphea*.

KEY WORDS *Cuphea*, western corn rootworm, *Diabrotica virgifera*

Developing new crops holds promise to diversify the agronomic landscape and may provide new strategies for pest control. Plants from the genus *Cuphea* have the potential to be developed as an oil seed crop to supply medium chain length fatty acids for several markets (Isbell 2002). Recent research explored the possibility of planting *Cuphea* as an alternative rotation crop [replacing soybean, *Glycine max* (L.) Merr.] to control western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae), but demonstrated only marginal control benefits over rotation with soybean (Behle and Isbell 2005). During two of the 4 yr of this research, large numbers of beetles were collected in emergence traps placed in plots planted to *Cuphea*, suggesting that western corn rootworm larvae might be able to complete development on the roots of this broadleaf plant. Peak catches by traps in *Cuphea* were recorded ≈4 wk later than peak catches by traps in corn plots. This observation was difficult to explain. Traps were in good condition, and catch numbers followed a smooth increase and decrease among traps placed in plots planted to *Cuphea,* and they were not the result of anomalous data of a single trap. As a result, this research was conducted to substantiate or discredit the hypothesis that western corn rootworm larvae successfully developed on this broadleaf plant. Herein, we report greenhouse and field research directed to document the ability of western corn rootworm larvae to use *Cuphea* as a host plant.

Materials and Methods

Greenhouse Larval Development. Survival of larvae feeding on *Cuphea* was evaluated using greenhouse-grown plants infested with western corn rootworm larvae from a nondiapausing laboratory colony (founders from USDA–ARS Brookings, SD, and maintained at USDA–ARS, Plant Genetics Research Unit, Columbia, MO) following techniques reported by Clark and Hibbard (2004). Two experiments were conducted. For the first experiment, *Cuphea* (PSR-23 = line selected from *C. viscosissma* Jacq. × *C. lanceolata* Ait.), corn (*Zea mays* L., Pioneer 34-B23, positive control), and sorghum [*Sorghum bicolor* (L.) Moench, 84G62, Pioneer Hi-Bred Inc., Johnston, IA], negative control) were compared. Seeds were sown in 15.2-cm (6-in.) pots containing 1:1 peat:soil-based

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[1] Corresponding author: Crop Bioprotection Research Unit, USDA–ARS–NCAUR, 1815 N. University Ave., Peoria, IL 61604 (e-mail: robert.behle@ars.usda.gov).

[2] Plant Genetics Research Unit, USDA–ARS, 205 Curtis Hall, University of Missouri, Columbia, MO 65211.

[3] New Crops and Processing Technology Research Unit, USDA–ARS–NCAUR, 1815 N. University Ave., Peoria, IL 61604.

Table 1. Number of live larvae and adults for a nondiapausing strain of western corn rootworm on three plants grown in a glasshouse

Crop	Exp 1		Exp 2		
	8 d	16 d	9 d	15 d	Adults
Cuphea PSR-23	0.0b	0.0b	0.0b	0.0b	0.0b
Cuphea (Mixed)			0.0b	0.0b	0.0b
Sorghum	1.0 ± 0.7b	1.2 ± 2.2b			
Pubescent wheat grass			4.3 ± 4.9b	2.7 ± 3.8b	1.3 ± 1.9b
Corn	7.8 ± 5.7a	13.4 ± 5.4a	13.5 ± 10.9a	6.8 ± 5.2a	5.7 ± 5.4a

Means in a column followed by the same are not significantly different ($P = 0.05$; LSD).

growing medium mixture (Professional General Purpose Growing Medium, Premier Horticulture Inc., Red Hill, PA). *Cuphea* seed was sown ≈1 wk before corn and sorghum seeds to allow time for slower growing *Cuphea* to develop sufficient root mass to be comparable with other test plants at the time of infestation. Ten pots were planted for each crop to provide five pots (replications) for each of two evaluation dates. Plants were grown in a greenhouse maintained at 25 ± 2.4°C. Six weeks after planting *Cuphea,* all pots were infested with 30 neonate western corn rootworm from the colony. At 8 and 16 d after infestation, five pots for each crop were randomly selected and placed individually in Tullgren funnels equipped with 60-W light bulbs for extraction of larvae. Emerging larvae were collected for 4 d.

A second experiment was conducted following the same technique, except as follows. Two *Cuphea* seed sources were used, PSR-23 and a mixture of *Cuphea* species from the USDA–ARS Plant Introduction Station in Ames, IA [*C. aperta* Koehne, PI 534838; *C. calophylla* Cham. & Schltdl., PI 534780, PI 566694, Ames 15482 ; *C. carthagenensis* (Jacq.), PI 534673; *C. glossostoma* Koehne, PI 534841; *C. glutinosa* Cham. & Schltdl., PI 534680; *C. heterophylla* Benth., PI 596717; *C. laminuligera* Koehne, PI 534900; *C. lanceolata* Aiton, PI 594945; *C. llavea* Lex., PI 534698; *C. lutea* Rose, Ames 17799; *C. procumbens* Ortega, PI 534708; *C. tolucana* Peyr., PI 534794, Ames 8134; *C. viscosissima* Jacq., PI 534726; and *C. wrightii* Gray, PI 561506). Control treatments were corn and pubescent wheatgrass, *Elytrigia intermedia* (Host), (Sharp Bros Seed, Clinton, MO), one of the better alternate hosts from previous experiments (Oyediran et al. 2004). Six replications were established. Each replication consisted of 16 total pots planted, four pots planted to each seed treatment. Of these four pots, one pot for each plant species was randomly selected to evaluate larval survival by using Tullgren funnels at 9 and 15 d after infestation and reporting the number of larvae recovered. Two remaining pots per plant species were caged to record for adult emergence.

2005 Field Plot: Urbana, IL. The research field used previously for evaluating *Cuphea* as a rotation crop for rootworm control (Behle and Isbell 2005) was again planted to corn and *Cuphea.* In 2005, each plot was 20 rows in width (space 0.76 m apart) by 9 m in length. Four treatment combinations consisted of corn (Pioneer 33G26) and PSR-23 *Cuphea* planted 4 May following corn and *Cuphea* crops of 2004. Treatments were replicated four times for a total of 16 plots. An adult emergence trap was placed in each plot on 20 June. Traps were those described by Behle and Isbell (2005) based on a design modified from Hein et al. (1985). Traps were sampled twice each week and the number and sex of the beetles were recorded.

Additionally, soil was collected from plots during the growing season and dissected for western corn rootworm. Three samples (each consisting of a shovel full of soil removed from 0 to 20 cm adjacent to the row of plants) were collected from corn and *Cuphea* plots. Samples were placed individually in plastic buckets, weighed, and dissected to search visually for rootworms. Samples were collected 20 June, 7 July, and 29 July, and the data are reported as the number of insects per kilogram of soil.

Statistical Analysis. Larval and adult data from greenhouse experiments were subjected to analysis of variance (ANOVA) by using the PROC GLM, SAS System for Windows, Release 8.00. The treatment means were separated by least significant differences (LSDs) using the LSD options with the Means statement (SAS Institute 1999). For greenhouse experiments, data for each sample date were analyzed separately. Data from emergence traps (beetles cage^{-1} d^{-1}) were subjected to ANOVA to evaluate the main effects of crop, sample date, and previous crop using the PROC GLM. Because the main effect of previous crop and all its interaction combinations were not significant ($P > 0.05$), the model was reduced to crop and sample date. In this case, the interaction effect was significant and the cage data to compare corn with *Cuphea* were analyzed separately for each collection date. Means were separated by LSD using the LSD options with the Means statement (SAS Institute 1999).

Results

Greenhouse Larval Development. First instars of western corn rootworm from a nondiapausing colony did not survive when used to infest greenhouse-grown *Cuphea.* No live larvae were found when sampled eight or 16 d after infestation (Table 1). Live larvae were found after infesting corn (positive control), which was significantly (experiment 1: 8 d, $F_{2,\ 12} = 8.26$; $P = 0.0055$; 16 d, $F_{2,\ 12} = 24.25$; $P < 0.0001$; experiment 2: 9 d, $F_{3,\ 20} = 6.80$; $P = 0.0024$; 15 d, $F_{3,\ 20} = 6.06$; $P = 0.0042$) greater than the number found infesting *Cuphea.* The number of larvae found in corn and their

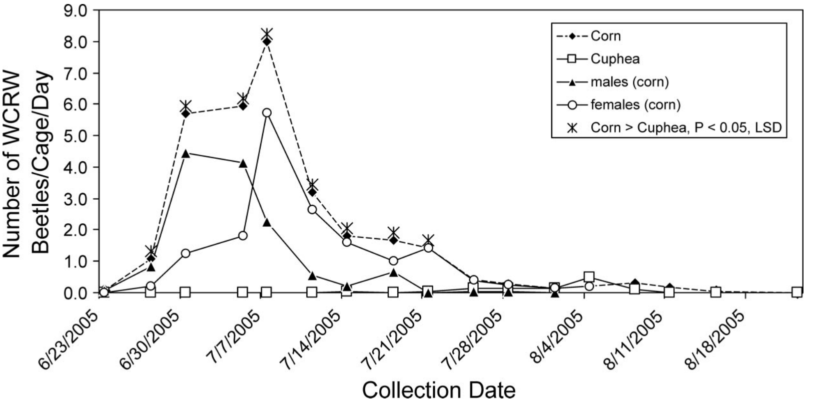

Fig. 1. Average number of adult western corn rootworm (WCRW) captured by emergence traps placed in field plots located near Urbana, IL, planted to corn and *Cuphea*. 28 of 29 beetles captured from *Cuphea* were female. Asterisk (*) denotes the number of beetles captured by traps in corn plots was significantly greater ($P < 0.05$) than those captured by traps in *Cuphea* plots.

size (data not presented) were appropriate based on previous experience using this technique of infestation. For the second experiment that included an additional source of *Cuphea* (mixed species accessions), the absence of adult emergence from *Cuphea* confirmed that western corn rootworm larvae from the nondiapausing colony did not survive when used to infest *Cuphea* plants. A few adults did emerge from pubescent wheat grass.

2005 Field Plot: Urbana, IL. Five pupae and 140 larvae were found in soil sampled from corn plots on 20 June, and this was the only sample that provided significant differences ($F_{1,\ 4} = 25.11$; $P = 0.0074$) between the two crops. By 7 July, five adults, four pupae, and 14 larvae were found in soil sampled from corn plots, indicating the maturation of the larval population. No rootworms were found in soil samples collected on 29 July from corn or in soil collected all three dates from plots planted to *Cuphea.* Emergence traps placed in corn plots showed a typical emergence pattern with a peak beetle emergence of eight beetles trap^{-1} d^{-1} on 8 July (Fig. 1). The total number of beetles captured by emergence traps placed in corn plots was 383 males and 430 females. Few beetles were captured by traps placed in *Cuphea* with almost none during July followed by a shallow peak (0.5 beetles trap^{-1} d^{-1}) on 4 August. The total number of beetles captured by emergence traps placed in *Cuphea* plots was 29; one male and 28 females.

Discussion

The high numbers of rootworm adults captured by emergence traps in *Cuphea* plots as reported by Behle and Isbell (2005) was unexpected because of the previous literature demonstrating the inability of rootworm larvae to survive on broadleaf plants. However, it seemed prudent to investigate the potential of this plant to serve as a suitable larval host after considering the ability of this insect to adapt to circumvent previously effective control tactics. All the data presented in this article support the conventional understanding that this broadleaf plant is not a suitable host for rootworm larval development. Controlled laboratory experiments using a nondiapausing strain and additional field evaluations (from the field where the original observation was recorded) provided no evidence to suggest that larvae can develop by feeding on roots of *Cuphea.*

The question still remains about the high numbers of beetles captured by emergence traps placed in plots planted to *Cuphea* (Behle and Isbell 2005). High numbers were captured only during 2 yr in which *Cuphea* plants grew vigorously and were blooming heavily during August when most of the beetles were captured. For less productive years, which include new data presented here, only a few beetles were captured by traps in *Cuphea* plots. It seems plausible that other factors may draw many beetles to the *Cuphea* plots in August as much of the corn crop begins to mature. *Cuphea* is an indeterminate plant that blooms continuously from mid-June until harvest or frost. When grown properly, the heaviest bloom occurs during August, a time when many beetles are ovipositing. The most vigorous *Cuphea* growth during the rotation study (2001–2004) was observed during the 2003 growing season, the year when the most beetles were captured by the emergence traps. It seems likely that the dense plant canopy combined with heavy bloom may have attracted beetles to the field while maintaining a moist soil surface, a condition suited for egg deposition. Kirk et al. (1968) showed that western corn rootworms prefer to oviposit in moist soil with weedy or trashy conditions (more similar to *Cuphea* plots) versus dry soil without weeds or trash (more similar to corn plots). We know that rootworms lay eggs in *Cuphea* from previous research that documented larval damage to corn planted in rotation after *Cuphea* (Behle and Isbell 2005). In these *Cuphea* plots, 28 of 29 beetles captured by emergence traps were female, which might suggest the beetles were captured after working through the soil to lay eggs. As yet, no direct evidence has been recorded to support this theory that the *Cuphea* plants specifically attract western corn rootworm adults.

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